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Polymer-*Paraburkholderia phytofirmans* PsJN Coated Diammonium Phosphate Enhanced Microbial Survival, Phosphorous Use Efficiency, and Production of Wheat

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Abstract: Low bioavailability of phosphorus (P) in alkaline/calcareous soils leads to low yields due to its precipitation/fixation with calcium (Ca). An effective method to enhance phosphorus use efficiency (PUE) and its availability to plants is the application of alginate bioaugmented coated di-ammonium phosphate (DAP). In this study, the pre-isolated P solubilizing *Paraburkholderia phytofirmans* (PsJN) coated with different concentrations of polymer (alginate) on DAP with and without carbon source was tested in incubation, pot, and field experiments to investigate microbial survival, Olsen P release, and its availability to plants. Results indicated that the maximum recovery of PsJN loaded on alginate at 30 days incubation with and without carbon source was 199×10^{10} and 82×10^{6} CFU mL^{-1} respectively. Application of alginate bioaugmented DAP released 962 mg kg^{-1} Olsen P after 60 days of incubation compared to 280 and 370 mg kg^{-1} Olsen P released by uncoated and simple alginate coated DAP, respectively. Results from pot trial revealed that application of 100% alginate bioaugmented DAP increased 26% plant height, 31% photosynthetic rate, 55% grain yield, and 6×10^{5} CFU g^{-1} root bacterial population, compared to uncoated DAP. Field trial results indicated that 22% grain yield, 14% straw yield, and 44% agronomy P efficiency were increased by applying 100% alginate bioaugmented DAP compared to uncoated DAP. This new approach resulted in controlled release of P from coated DAP that not only reduced phosphorus fixation but also enhanced the bioavailability of P to plants.

Keywords: polymer; carrier; plant–microbe interaction; control release; phosphorus; wheat; yield

1. Introduction

Fertilizers are the most important and expensive inputs in agricultural production. It will be a great challenge to increase crop productivity to fulfill the food demand over the next 50 years in the presence of increasing population, decreasing arable land, rapid global climate change, increasing water scarcity, and the rising price of agriculture inputs. The role of fertilizers in this scenario has gained increasing importance to get high agricultural production per unit area from the same soils,

with declining soil fertility. Furthermore, high cereal production is only possible through the wise use of fertilizers. Currently, 40–60% of cereal production depends on fertilizers and by 2050, almost 110% grain production will have to depend on fertilizers. Phosphorus (P) is one of the most important macronutrients for optimal crop production and its deficiency in soil limits the growth and yield of agricultural crops [1–3]. There is a significant difference between the externally applied P and its uptake by plants, indicating only a small proportion of P fertilizer applied to soil is utilized by the plants. Consequently, 80–90% of applied P remained in the soil either by fixation or precipitation with Ca in calcareous soils [2–4]. This loss of applied P results in not only yield losses and enhanced production costs, but also to a severe environmental impact as an important constituent of eutrophication.

Unavailability of P from applied P fertilizers to plants can be prevented by using a number of techniques including acid application, band placement, genetic engineering and biotechnological techniques, selection of efficient crop varieties, and impregnation of phosphatic fertilizers with P solubilizing bacteria [3,5,6]. Besides, any other technique that can be helpful to increase the efficiency of applied P fertilizers in a cost-effective manner will be popularized among agriculturists. No doubt, some agronomic practices and use of P solubilizing microbes have attained importance in some segments [6,7], however, application of these approaches on large scale in all conditions is still under question. More recently, the coating of fertilizer granules with polymers has drawn attention for enhancing fertilizer use efficiency [3,8].

The most important function of polymer coated fertilizer is to release nutrients gradually according to plant requirement which otherwise precipitated or fixed due to high calcium activity in calcareous soils [3,9]. Plant growth promoting endophytes (PGPEs) are also one of the most popular choices for increasing availability and phosphorus use efficiency (PUE) for plants by assisting in the hydrolysis of P particles in small fractions which facilitates absorption and assimilation by crops [6,10]. Moreover, endophytes have abilities to mobilize fixed P compounds hoarded in soil via exchange reactions; chelation, acidification, and phytohormones production on one hand while on the other improve root growth for effective nutrients absorption [6,11,12]. This approach permits crops to uptake both applied as well as fixed P. Therefore, the ultimate goal of this approach may be less application of phosphatic fertilizers.

One of the challenges of using PGPEs is the successful delivery and maintenance of microbial survival in the rhizosphere. Various biotic and abiotic stresses in soil influence and retard bacterial colonization on plant roots [13]. Previously, peat and clay were used as carriers for inoculants which have high biochemical changes properties and less cells viability/existence at storing environments [14]. Recently, it has been reported that microbial viability can be improved by immobilizing into biodegradable polymer carriers (natural and synthetic polymers) for longer time. Numerous advantages related to the immobilization of microbes in polymers are conferred such as protection, slow release, prolonged storage, and easy transportation [15].

Previously studies indicated improved PUE via seed inoculation under controlled conditions [14,16] but these types of approaches have limited scope. The current study was conducted to further enhance the PUE and wheat production compared to our previous experiment where alone polymer coated DAP was used [3]. Since there is a lack of information regarding the effectiveness of combined coatings on DAP, it is imperative to determine whether it could act as a new, more sustainable fertilizer for addressing plant P demands. This comprehensive study was conducted with following objectives: (1) to check the viability of bacteriaum *Burkholderia phytofirmans* PsJN in prototype solution with different concentrations and carbon sources, (2) to determine the release profile of P from DAP with varying level of temperature, and (3) to investigate the effect of polymer-bioaugmented bacterial coated DAP on growth, yield, and PUE of wheat.

2. Materials and Methods

2.1. Description of Laboratory Experiments

Endophyte *Paraburkholderia phytofirmans* strain PsJN wild type and its derivative *gusA*10 labelled were collected from Soil Microbiology and Biochemistry Laboratory, Institute of Soil and Environmental Sciences (ISES), University of Agriculture, Faisalabad (UAF) for these experiments. Strain PsJN is among the most studied bacterial endophyte so far and is able to establish rhizospheric and endophytic populations associated with a variety of plant species. Originally isolated from surface-sterilized Glomus vesiculiferum-infected onion roots, strain PsJN has been shown to colonize a wide variety of plants (e.g., potato, tomato, wheat, maize and grapevines [16,17]) and it stimulates plant growth and vitality in many of its host plants due to variety of its beneficial growth promoting traits e.g., hormone production, nutrient solubilization, ACC-deaminase and siderophore production under lab and greenhouse conditions. Inoculum of the selected strains was prepared in LB broth containing spectinomycin (100 g mL^{-1}) and adjusted uniform population of bacteria 10^8–10^9 CFU^{-mL} for inoculation [17,18]. Selected endophytic strain i.e., *Paraburkholderia phytofirmans* PsJN inoculum and polymer (Na-alginate) solution was prepared by mixing polymer in inoculum in such a manner that concentration of each polymer remained constant. These prototype formulations [(Polymer (Na-alginate) concentration (0.5%, 1% and 1.5%) + PsJN] were stored at room temperature in sterile falcon tubes for subsequent evaluation of the survival of microbes for one month. The effects of formulations (polymer + endophytic bacteria) on the bacterial viability was estimated by preparing serial dilutions in a buffer solution and plating 100 µL of each dilution on selective LB medium containing spectinomycin (100 g mL^{-1}), 5-bromo-4-chloro-3-indolyl-d-glucuronide (XGlcA) (100 g mL^{-1}), and isopropyl-d-galactopyranoside (IPTG) (100 g mL^{-1}) as described by Naveed et al. [16]. Plates were placed in incubator at 28 ± 1 °C for 48–72 h. Colonies showing blue color were counted by using colony counter as recommended by Naveed and Compant et al. [16,17]. The best concentration of alginate selected from this experiment was 1.5%. This concentration plus microbe prototype solution was enriched with carbon source (1% glucose (G), 1% glycerol (Gly), and combination i.e., 1% G + 1% Gly) and microbial survival rate was measured over time [17]. Prototype solution (alginate 1.5%) + (1% G + 1% Gly) + PsJN) and alone polymer at 1000 mL was coated on 50 kg diammonium phosphate (DAP) fertilizer under laboratory conditions [3]. The coated DAP grains were dried and stored at room temperature till use. Microbial survival was also measured on coated DAP grains stored at different temperatures (10, 25, and 40 °C) over different time interval. For these measurements 1-g coated DAP was dissolved in saline buffer solution and serial dilution was made and spread on amended LB plates placed in incubator for 72 h at 28 °C as described by Naveed et al. [16].

Disposable cups were filled with 200 g ground, dried, and sieved soil collected from Research Farm, Institute of Soil and Environmental Sciences. Di-ammonium phosphate (DAP) i.e., 1 g 100 g^{-1} of soil in the form of polymer-entrapped bacteria coated, simple (alone) polymer coated and uncoated was mixed thoroughly into 200 g soil before adding moisture. The weight of cups after adding fertilizer, soil, and water was recorded and maintained after every 24 h by distilled water.

Soil samples weighing 3 g collected from cups were extracted with 0.5 M $NaHCO_3$ solution adjusted to pH 8.56. A total of 5 mL of clear filtrate was taken in a 100 mL volumetric flask and then 5 mL color developing regent was added (ascorbic acid). Volume was made up to the mark. Reading was recorded on a spectrometer (T80 UV/VIS Spectrometer) using 880 nm wavelength and with the help of a standard curve. Available phosphorus was determined after 15, 30, 45, and 60 days by method as described by Noor et al. [2]. Five grams of soil was taken from the cup treated with DAP [(coated DAP + PsJN)] to determine the microbes release pattern from coated DAP into 15 mL saline buffer solution (0.9% *w/v* NaCl) and put in a shaking incubator at 180 rpm for 30 min. After settling of soil particles, serial dilutions were prepared, spread on LB plates, placed in incubator for 4 days at 28 ± 2 °C following the method described by Naveed et al. [16].

2.2. Description of Pot Experiment

A pot experiment was conducted during 2018–2019 to investigate the efficacy of endophytic bacterium *Paraburkholderia phytofirmans* PsJN on polymer bioaugmented microbes coated DAP. Effect of this treatment was also investigated on growth, yield, and phosphorus use efficiency of wheat. The soil used during the experiments was taken from Research Farm, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. However, 10 kg soil was filled in each pot. The soil belonged to Lyallpur Soil Series and sandy clay loam in texture with pH 7.4, organic matter 7.5 g kg^{-1}, and Olsen's P 6 mg kg^{-1} soil. However, average rain fall during the growing season was 91 mm and temperature varies from 4.4 to 27.70 °C. Recommended rates of N, P, and K fertilizers (1.304, 0.978, 0.500 g pot^{-1}) were applied in the form of urea, DAP, and sulfate of potash (SOP). Moreover, all doses of P and K and N (1/3rd dose) fertilizers were homogeneously mixed in topsoil during crop sowing but the remaining N dose was applied in two splits at 45 and 60 days of crop sowing. In all treatments, N and K fertilizers were applied at recommended rates while polymer bioaugmented microbes coated DAP was applied as 50%, 75%, and 100% of the recommended rate of DAP applied along with treatments of alone polymer coated DAP and uncoated DAP for comparison between coated DAP and conventional DAP application. Treatments were arranged under completely randomized design with six replications. Six seeds of wheat variety (Faisalabad-2008) were sown in each pot. Four plants of the same vigor in each pot were retained after two weeks of successful germination. Uprooted seedlings were chopped and incorporated in the same pot. At 45 days of germination, three replications were harvested for microbial enumeration and the remaining replications were sustained till maturity (120 day after sowing) for determination of growth, physiological, and chemical parameters. The pots were irrigated with tap water according to crop water requirement with constant agronomic and protective measures for wheat production in all treatments. Plants were removed from the pots (at 45 days of germination) to obtain the rhizosphere soil and root samples. Roots were gently agitated and the soil still attached with root was collected. For detection of rhizospheric bacteria, 5 g of soil was taken into 15 mL saline buffer solution (0.9% *w*/*v* NaCl) and put in a shaking incubator at 180 rpm for 30 min. After settling of soil particles, serial dilutions were prepared, spread on LB plates amended with spectinomycin, XGlucA, and IPTG, placed in incubator for 4 days at 28 ± 2 °C. For root colonization, 2 g of surface sterilized roots were homogenized with a sterile mortar and pestle in 15 mL saline buffer solution, and placed in a shaker at 180 rpm for 30 min. The homogenates were serially diluted and cultured on LB plates. Bacterial colonies were counted after 72 h of incubation at 28 ± 2 °C following the method described by Naveed et al. [16]. At vegetative stage (before tasselling) the parameter i.e., photosynthetic rate (A) was measured by using CIRAS-3 (PP System, Amesbury, MA, USA) with PLC3 universal leaf cuvette, measuring both sides of the fully expanded upper leaf. Cuvette was provided light via light emitting diodes (LED) and with a photon flux of 1000 µmol m^{-2} s^{-1}, ambient leaf temperature, and 390 µmol mol^{-1} CO_2. Chlorophyll content in flag leaves was measured with the help of a portable Chlorophyll Meter SPAD-501 at tasselling stage and measured for comparison of different treatments. The chlorophyll contents and physiological parameter (photosynthesis rate) were taken as described by Noor et al. [2]. Data regarding growth and yield attributes were recorded at different crop growth stages through standard procedure. For chemical analyses, shoots and grains of the harvested plants were digested by the method of Wolf [19]. The dried and ground shoot or root material (0.1 g) was digested with sulphuric acid and hydrogen peroxide according to the method of Wolf (1982). For this purpose, the dried ground material (0.1 g) was placed in digestion tubes, 2 mL of concentration H_2SO_4 was added and incubated overnight at room temperature. Then, 1 mL of H_2O_2 (35% A.R) was added, tubes were ported in a digestion block and heated up to 350 °C, unit fumes were produced, and continued to heat for another 30 min. Digestion tubes were removed from the block and cooled. Then, 1 mL of H_2O_2 was slowly added and tubes were placed back on to the digestion block until fumes were produced for 20 min. Again, digestion tubes were removed. The above step was repeated until the cooled material became colorless. The volume of extracts was made up to 50 mL with distilled water. Then, it was filtered and used for determination of mineral elements. Nitrogen, phosphorus, and

potassium concentration was determined [20]. For nitrogen, a digested 10 mL aliquot was taken in a Kjeldahl flask for distillation having 20 mL 40% NaOH. Nitrogen in the form of ammonia was captured in 4% boric acid with indicator bromocresol and titrated against 0.01 N standard sulphuric acid till pink end point. Phosphorus concentration in digested samples was determined by using vanadate molybdate spectrophotometer. Digested filtrate potassium was analyzed using a flame photometer (410 Sherwood) through the method of [20]. Moreover, total phosphorus uptake was calculated by Noor et al. [2]. P efficiency parameters (agronomic and recovery efficiency) were estimated by following methods described by Noor et al. [2]. Various forms of P use efficiency were determined using the following formulae.

$$\text{AE (g grains/g fertilizer)} = \frac{\text{P fertilized pot yield} - \text{P unfertilized pot yield}}{\text{Amount of fertilizer applied}}$$

$$\text{RE (\%)} = \frac{\text{Total P uptake byfertilized plant} - \text{Total P uptake byunfertilized plant}}{\text{Amount of fertilizer applied}} \times 100$$

AE = agronomic efficiency, RE = agronomic recovery efficiency, Total P uptake = P uptake in grains + P uptake in straw.

2.3. Description of Field Experiment

Field experiment was conducted during 2018–2019 at the research area, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan which was situated on global position at 73° East longitude, 31° North latitude and 184 m above from sea level having semiarid climate. Soil physico-chemical properties were the same as mentioned above in the pot experiment. Similarly, a treatment plan was applied as described earlier in the pot experiment to evaluate the success of the approach under natural field conditions. The field was ploughed three times followed by planking. Layout was made according to randomized complete block design by assigning an area of 4×4 m to each plot with three replications. Seeds of wheat variety Faisalabad-2008 were sown at the rate of 120 kg ha^{-1} through manual drill in each experimental unit. Recommended rates of N, P, and K fertilizers (120, 90, 60 kg ha^{-1}) were applied in the form of urea, DAP, and sulfate of potash (SOP). Furthermore, the recommended rate of P and K fertilizers were broadcasted in soil during crop sowing, but N dose was applied in three splits at 25, 45, and 60 days of crop sowing. Cultural and irrigation practices were kept the same for all the treatments. Crop was irrigated with canal water five times during the growing period. Chlorophyll contents were taken after 70 day of sowing from full expended flag leaf chlorophyll meter, respectively. Crop was harvested 120 days after sowing. One square meter area was randomly selected from each plot for measuring growth and yield attributes before harvesting and average values were computed. Grain and straw yields were recorded at harvest from each plot for total productivity calculation. Grains and straw samples were digested by the method of Wolf [19] and their P contents were determined by the method of Chapman and Pratt [20]. However, total P uptake and P efficiencies were computed by method described Noor et al. [2].

2.4. Statistical Analysis

Data regarding different parameters in present study was analyzed by Steel et al. [21] by using analysis of variance and Tukey's test was used for ranking the mean differences by using Statistix 8.1 software at 5% probability level. The means and standard errors were calculated using Microsoft Excel 2010.

3. Results

3.1. Laboratory Experiments

3.1.1. Microbial Survival in Polymer Prototype Matrix and after Coating on DAP Fertilizer Surface

All the three concentrations (0.50%, 1.00%, and 1.5%) in polymer prototype matrix-maintained viability of PsJN, as is depicted in Figure 1A. Although these concentrations performed in the same

order of magnitude, however, concentration of 1.50% was found relatively better in terms of microbial survival. The effect of other concentrations on cell viability can be ranked as 1% and 0.5%, respectively, after 1.5% concentration. The stationary phase was observed in all treatments at 15 days of incubation but afterward at 30 days, an increasing trend was detected in CFU^{-mL} which is also obvious from Figure 1A. At 15 days incubation, maximum bacterial number of PsJN ($79 \pm 1.77 \times 10^6$ CFU^{-mL}) was observed in prototype polymer solution. However, this count was increased to $82 \pm 2.51 \times 10^6$ CFU^{-mL} of PsJN at 30 days of incubation. No doubt treatment of 1% polymer also supported microbial viability with number of cells of $72 \pm 2.87 \times 10^6$ CFU^{-mL} of PsJN at 15 days but it had a declining trend in microbial survival with a record of $70 \pm 1.57 \times 10^6$ CFU^{-mL} for PsJN after 30 days of incubation. Minimum survival count was observed in the treatment of 0.50% polymer concentration i.e., $69 \pm 2.23 \times 10^6$ CFU^{-mL} PsJN at 15 days of incubation. However, at 30 days incubation cell viability of PsJN recorded was $64 \pm 1.92 \times 10^6$ CFU^{-mL}.

The above results highlight better performance of 1.5% concentration of alginate. This selected concentration of polymer was further investigated for microbial survival after amending with organic carbon sources to find the most suitable combination for the preparation of polymer-based inoculant. The criterion followed was recording of optimum survival rate of endophytic bacterial sp. under investigation. Data regarding microbial viability at 30 days of incubation indicate maximum viability of bacterial sp. PsJN ($199 \pm 1.87 \times 10^{10}$ CFU^{-mL}) polymer prototype solution of alginate (1.5%) with combination of 1% glucose (G) + 1% glycerol (Gly). It was followed by alginate (1.5%) with 1% G ($152 \pm 3.86 \times 10^{10}$ CFU^{-mL} of PsJN) at 30 days of incubation period as shown in Figure 1B. Like 15 days of incubation period, minimum survival rate was observed in the alginate concentration of 1.5% amended with 1% concentration of Gly. This treatment showed microbial survival of PsJN up to $125 \pm 2.76 \times 10^{10}$ CFU^{-mL} at 30 days of incubation.

Another experiment was conducted to test the shelf life/storage period for microbe survival on polymer-bioaugmented PsJN coated DAP surface. Polymer prototype solution [1.5% alginate + (1% G + 1% Gly) + PsJN] coated DAP fertilizer was stored at different temperatures for microbial survival test. A more rapid decline in cell viability at high storage temperature was noted, as compared to low temperature of 10 °C (Figure 1C). At 10 °C temperature, viability was best maintained which showed stable values at 1, 15, 30, 60, and 90 days of incubation as $46 \pm 3.21 \times 10^7$, $57 \pm 2.69 \times 10^7$, $61 \pm 3.46 \times 10^7$, $66 \pm 4.08 \times 10^7$, and $55 \pm 2.59 \times 10^7$ CFU^{-g} fertilizers, respectively. The sample stored at 25 °C also revealed a similar trend like 10 °C stored samples but slightly inferior in its ability to conserve viability of both microbes at 90 days of storage period ($13 \pm 1.10 \times 10^7$ CFU^{-g} fertilizers PsJN). In contrast to these results, viability was dropped by about one order of magnitude after three months when samples were stored at 40 °C. Maximum viability was recovered at the first day for PsJN ($67 \pm 3.4 \times 10^7$ CFU^{-g} fertilizer), afterwards, a declining trend was recorded up to the end of the experiment. Minimum viability was observed in the treatment stored at 40 °C which was $9 \pm 0.70 \times 10^7$ CFU^{-g} DAP fertilizer (Figure 1C).

3.1.2. Microbes and Phosphorus Release Pattern from Coated DAP in Soil

Significant results were recorded for microbial release pattern under controlled conditions. Data in Figure 1D showed linear increase in cell number up to two months (60 days) of incubation time. Though, results revealed a minor decline in cell number at 60 days of incubation, but log cell number remained constant. The recovery of microbes was maximum ($112 \pm 3.32 \times 10^6$ CFU^{-g} soil) from soil treated with polymer-bioaugmented PsJN coated DAP at 45 days of incubation at 25 °C. It was followed by 30 days of incubation time where count was $85 \pm 3.32 \times 10^6$ CFU^{-g} soil. However, at 60 days of incubation a decline in population occurred with a count of $77 \pm 3.32 \times 10^6$ CFU^{-g} soil PsJN population. These results depict the microbes release pattern in a controlled manner.

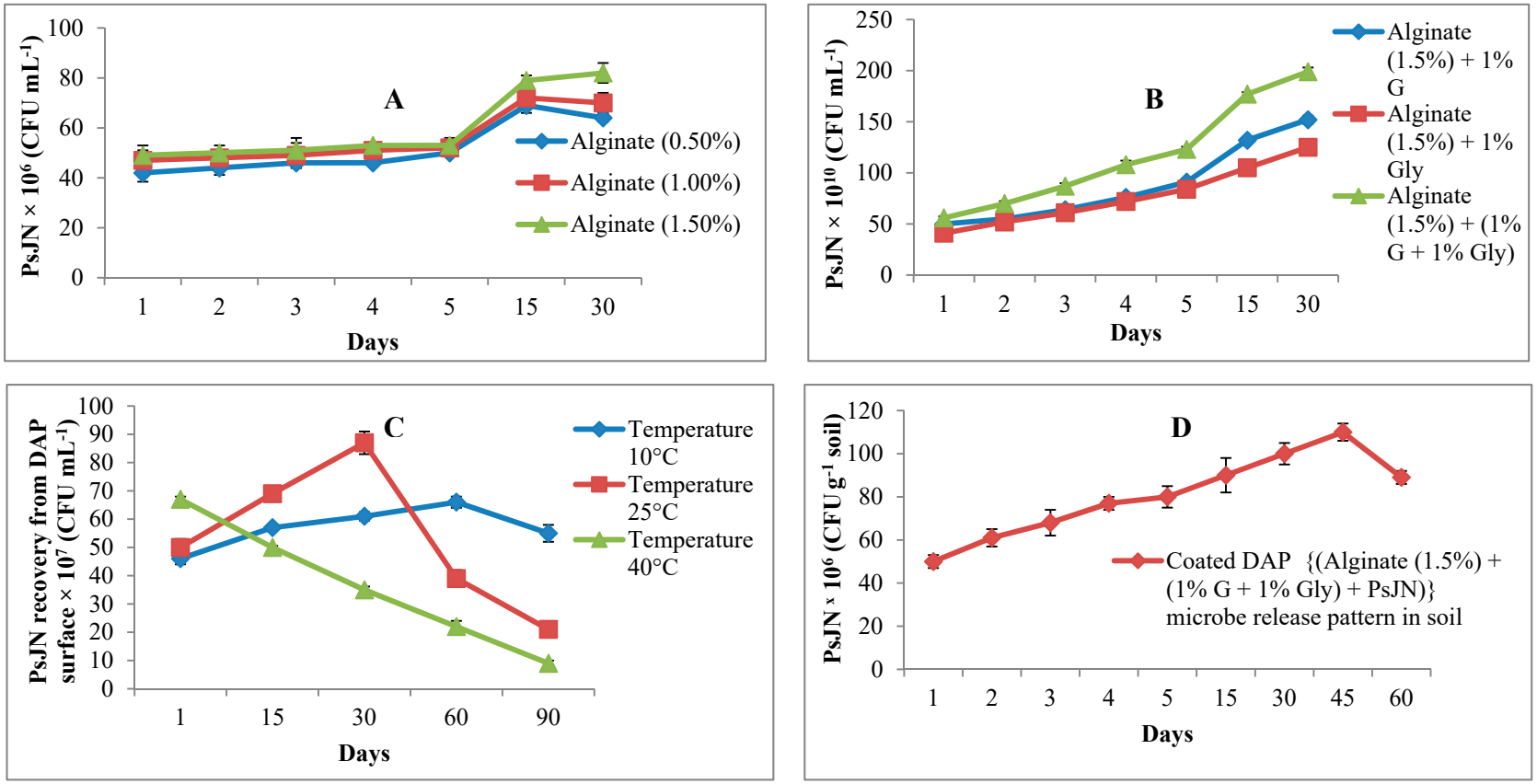
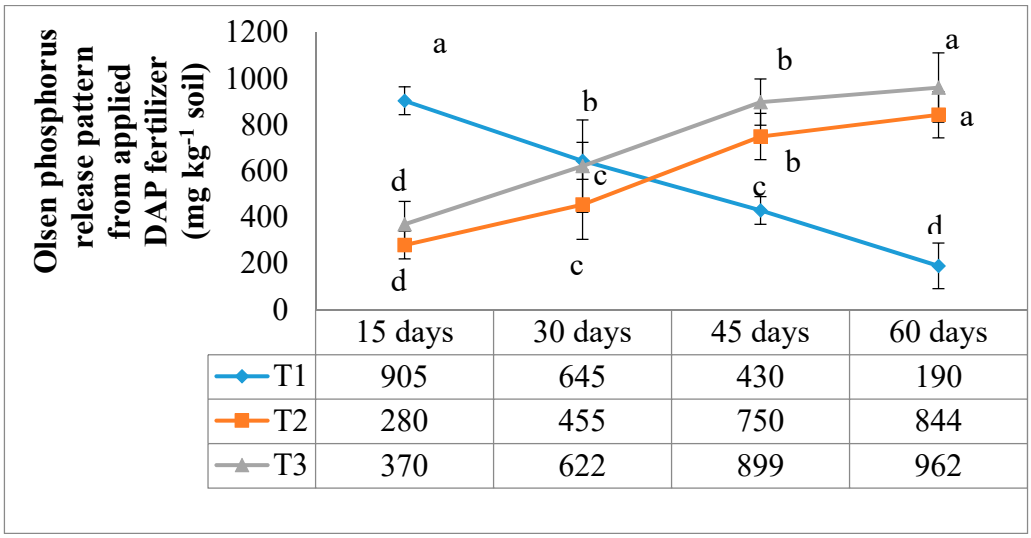


Figure 1. Viability of endophytic bacterium *Burkholderia phytofirmans* PsJN in (**A**) different concentrations of polymer prototype solution, (**B**) selected concentration of polymer prototype solution amended with carbon sources, (**C**) recovery of PsJN from coated DAP surface stored at different temperatures, and (**D**) release pattern of PsJN from coated DAP in soil. Note: G—glucose; Gly—Glycerol; CFU—colony forming unit, $n = 3$.

Data in Figure 2 explain the effect of coated and uncoated DAP fertilizer on Olsen P release in soil at 100% of field capacity moisture level at different incubation time intervals. Relatively high release of P was recorded in soil treated uncoated DAP fertilizer (905 mg kg^{-1} soil) at 15 days of incubation time compared with all other incubation time intervals and coated DAP fertilizer treatments. Vice versa of this was significantly lower release of P in soil treated with polymer coated DAP fertilizer. This slow/less release of P in soil might be due to the presence of a layer of polymer on coating DAP fertilizer. At 15 days of incubation, comparison among polymer treatments elucidated maximum Olsen's P release (280 and 370 mg kg^{-1} soil) in soil treated with alone polymer and polymer-bioaugmented PsJN coated DAP fertilizer. However, this trend of P release was quite different after 30 days of incubation, where P release in soil was decreased significantly in the treatment of uncoated DAP fertilizer compared to already shown high P release in soil at 15 days of incubation. Alone polymer and polymer bioaugmented/entrapped microbial coated DAP fertilizer showed an increase in P release trend with increase in the time of incubation intervals. However, this trend was more or less equal at 45 and 60 days of incubation time periods. At 60 days of incubation maximum Olsen's P (962 mg kg^{-1} soil) was released in soil treated with polymer-bioaugmented PsJN coated DAP compared to uncoated DAP (190 mg kg^{-1} soil Olsen's P). Polymer-bioaugmented PsJN coated fertilizer showed an increase in soil P release compared to alone polymer coated and uncoated DAP fertilizer.



	15 days	30 days	45 days	60 days
T1	905	645	430	190
T2	280	455	750	844
T3	370	622	899	962

Figure 2. Phosphorus release pattern in soil treated with coated and uncoated DAP fertilizer at field capacity moisture level over different time intervals of incubation. Note: Values sharing the same letter did not differ significantly at 5% level of significance. DAP—diammonium phosphate; T1—uncoated DAP; T2—alone polymer coated DAP; T3—polymer-bioaugmented PsJN coated DAP, $n = 3$.

3.1.3. Effect of Polymer-Bioaugmented PsJN Coated DAP on Wheat Growth and Yield Contributing Parameters under Wire-House Conditions

Polymer-bioaugmented PsJN coated DAP fertilizer treatment showed more promising results than uncoated and alone polymer coated DAP fertilizer treatments regarding growth and yield attributes of wheat (Table 1). Polymer-bioaugmented PsJN coated DAP application at recommended rate caused a noteworthy upsurge in plant height (27%) over uncoated DAP fertilizer treatment (Table 1). Likewise, when root biomass per pot was calculated of wheat plants, treatment T_4 (Polymer-bioaugmented PsJN coated DAP) gave 29% increase over uncoated DAP T_2 that differed significantly, however, it was also significantly different from other treatments (Table 1). It is clear from data that improvement in number of fertile tillers was 75% higher in the treatment receiving polymer-bioaugmented PsJN coated DAP fertilizer at recommended rate as compared to uncoated DAP (Table 1). Up to 30% and 19% increase in chlorophyll contents was recorded in the treatments of polymer entrapped

PsJN and alone polymer coated DAP, respectively, as compared with uncoated DAP. Coated DAP fertilizer effect on photosynthesis rate was also more prominent (31% and 18.5% higher than uncoated DAP fertilizer treatment) where wheat plants received P from recommended to reduced rates of polymer-bioaugmented PsJN coated DAP i.e., 100% and 75% of the recommended rate, respectively. Polymer-bioaugmented PsJN coated DAP application to wheat at 100% of the recommended rate enhanced 1000 grains weight 17.50% as compared to uncoated DAP treatment. Application of the recommended rate of polymer plus PsJN coated DAP enhanced wheat grains yield 55.50% over uncoated DAP. In the case of 75% and 50% of the recommended rate of polymer-bioaugmented PsJN coated DAP application, increase in grain yield over uncoated/commercial DAP treatment was 33% and 6%, respectively. However, results were more conspicuous in straw yield pot^{-1} by 31%, 27%, and 4% higher than uncoated treatment where 100%, 75%, and 50% of the recommended rate of polymer-bioaugmented coated DAP were applied to wheat, respectively.

Microbial delivery at the target site from coated DAP in soil was also determined. Significant variation in microbial (PsJN) number in rhizospheric soil and root endosphere colonization was observed among 100%, 75%, and 50% recommended rate of polymer-bioaugmented PsJN coated DAP treatments. Microbial enumeration results regarding rhizosphere soil and root endosphere showed that 100% recommended rate of polymer-bioaugmented PsJN coated DAP recovered a greater number of cells i.e., rhizospheric soil (8×10^7 CFU^{-g} soil) and root endosphere (6×10^5 CFU^{-g} root) over reduced rate receiving treatments (Table 1).

3.1.4. Effect of Polymer-Bioaugmented PsJN Coated DAP on Nutrients Concentration in Wheat Produces under Wire-House Conditions

Application of polymer-bioaugmented PsJN coated DAP extraordinarily affected the absorption of nutrient concentration in grains and straw of wheat (Table 2). Polymer-bioaugmented PsJN coated DAP at the recommended rate caused increased nitrogen concentration in grains and straw of wheat and showed 36% and 31% more increase in nitrogen concentration in grains and straw, respectively, as compared to uncoated DAP. Treatment of 75% of the recommended rate of polymer-bioaugmented PsJN coated DAP increased nitrogen concentration in grains (25%) and straw (18%) over uncoated DAP treatment. Comparison between alone polymer and polymer-bioaugmented PsJN coated DAP treatments at the recommended rate, polymer-bioaugmented PsJN coated DAP treated plants showed 12% and 22% increase in nitrogen concentration of grains and straw, respectively, over alone polymer coated DAP treated plants. Data on phosphorus concentration of grains and straw showed that application of alone polymer coated DAP fertilizer increased phosphorus concentration in grains and straw by 16% and 11%, respectively, compared to uncoated DAP. Phosphorus concentration in grains and straw was further increased by PsJN bioaugmented polymer coated DAP treated treatment where 48% and 33% increase in grains and straw P concentration was recorded in the treatment receiving 100% of the recommended rate of polymer-bioaugmented PsJN coated DAP, respectively, as compared to uncoated DAP. However, potassium grains (29%) and straw (37%) were higher in the treatment receiving the recommended rate of polymer-bioaugmented PsJN coated DAP than the recommended rate of uncoated DAP. In the case of treatment comparison of the recommended rate of polymer-bioaugmented PsJN with alone polymer coated DAP, estimated increase in grains and straw potassium was 15% and 18%, respectively, over the recommended rate of alone polymer coated DAP treatment.

Table 1. Effect of polymer-bioaugmented PsJN coated DAP on yield contributing attributes of wheat and microbe delivery at the target site in a pot experiment.

Treatments	Plant Height (cm)	Root Biomass (g pot^{-1})	Number of Fertile Tillers (pot^{-1})	Chlorophyll Contents (SPAD)	Photosynthetic Rate (μmol m^{-2} s^{-1})	1000 Grains Weight (g)	Grains Yield	Straw Yield	Rhizospheric Soil PsJN Recovery	Root Endosphere PsJN Recovery
							(g pot^{-1})		CFU^{-g} Soil	CFU^{-g} Root
T_1 (control)	54 f	23 f	5 f	33 e	9 f	31 f	5 f	8 f	—	—
T_2	68 de	34 d	8 de	43 d	16 d	40 d	18 de	26 de	—	—
T_3	75 c	39 bc	11 bc	50 bc	18 bc	43 bc	21 c	34 bc	—	—
T_4	86 a	44 a	14 a	56 a	21 a	47 a	28 a	36 a	8×10^7 a	6×10^5 a
T_5	78 b	40 b	11 ab	51 b	19 b	44 b	24 b	33 b	4×10^6 ab	8×10^4 ab
T_6	69 d	33 de	9 d	43 d	14 e	38 de	19 d	27 d	6×10^4 c	3×10^2 c
HSD	4.2	3.31	1.98	3.21	1.56	2.89	1.56	2.30	—	—

Note: Values sharing the same letter(s) did not differ significantly at 5% level of significance. T_1—control; T_2—uncoated DAP; T_3—alone polymer coated; T_4—polymer-bioaugmented PsJN coated DAP (100% of recommended rate); T_5—polymer-bioaugmented PsJN coated DAP (75% of recommended rate); T_6—polymer-bioaugmented PsJN coated DAP (50% of recommended rate), $n = 3$.

Table 2. Effect of polymer-bioaugmented PsJN coated DAP on nutrient concentration of wheat in a pot experiment.

Treatments	Grains			Straw		
	Nitrogen	Phosphorus	Potassium	Nitrogen	Phosphorus	Potassium
	(%)			(%)		
T_1 (control)	1.23 f	0.10 f	0.82 f	1.01 f	0.08 f	1.08 f
T_2	1.74 de	0.25 e	1.10 de	1.52 d	0.18 e	1.45 e
T_3	2.04 c	0.29 c	1.23 bc	1.63 c	0.20 cd	1.68 c
T_4	2.38 a	0.37 a	1.42 a	1.99 a	0.24 a	1.98 a
T_5	2.18 b	0.32 b	1.30 b	1.79 b	0.23 ab	1.82 b
T_6	1.82 d	0.27 d	1.11 d	1.49 de	0.21 c	1.59 cd
HSD	0.18	0.03	0.09	0.11	0.012	0.12

Note: Values sharing the same letter(s) did not differ significantly at 5% level of significance. T_1—control; T_2—uncoated DAP; T_3—alone polymer coated; T_4—polymer-bioaugmented PsJN coated DAP (100% of recommended rate); T_5—polymer-bioaugmented PsJN coated DAP (75% of recommended rate); T_6—polymer-bioaugmented PsJN coated DAP (50% of recommended rate), $n = 3$.

3.1.5. Effect of Coated DAP on Phosphorus Efficiency of Wheat under Wire-House Conditions

Phosphorus efficiency was estimated in terms of recovery efficiency and agronomic efficiency of phosphorus applied by coated DAP over uncoated DAP treatments. As expected, the P recovery and agronomic efficiency of the applied P through coated DAP was increased as the level of application reduced as compared to recommended rate (Figure 3). Maximum upsurge in P recovery efficiency was estimated in treatment where polymer-bioaugmented PsJN coated DAP was applied at half of the recommended rate, it was 110% more than that of uncoated DAP treatment. Application of polymer-bioaugmented PsJN coated DAP at 75% the recommended rate enhanced P recovery efficiency up to 100%, followed by 100% the recommended rate of polymer-bioaugmented PsJN coated DAP treatment which improved P recovery efficiency 95% over uncoated DAP. As far as P agronomic efficiency is concerned, polymer-bioaugmented PsJN coated DAP significantly increased P agronomic efficiency compared to uncoated DAP treatment. Furthermore, up to 37% and 89% increase in P agronomic efficiency was recorded in treatment alone polymer and polymer-bioaugmented PsJN coated DAP as compared with uncoated DAP, respectively. However, the treatment effect was more conspicuous in P agronomic efficiency by 127% and 111% higher than recommended uncoated treatment where polymer-bioaugmented PsJN coated DAP at 50% and 75% recommended rate were applied to wheat plants, respectively.

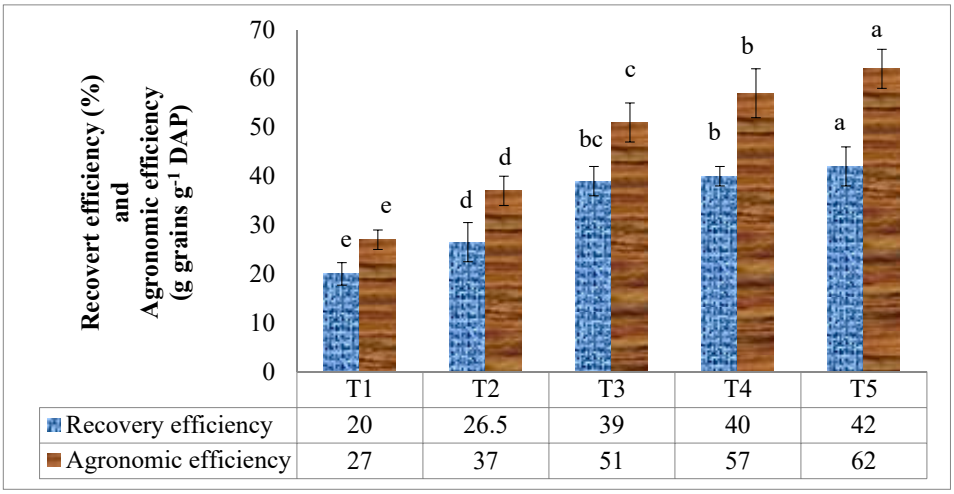


Figure 3. Efficacy of polymer-bioaugmented PsJN coated DAP on phosphorus use efficiency parameters of wheat in a pot experiment. Note: Values sharing the same letter(s) did not differ significantly at 5% level of significance. RE—recovery efficiency of phosphorus; AE—agronomic efficiency of phosphorus; DAP—diammonium phosphate; T1—uncoated DAP at 100% recommended rate; T2—alone polymer coated DAP at 100% recommended rate; T3—polymer-bioaugmented PsJN coated DAP at 100% recommended rate; T4—polymer-bioaugmented PsJN coated DAP at 75% of recommended rate; T5—polymer-bioaugmented PsJN coated DAP at 50% of recommended rate, $n = 3$.

3.1.6. Effect of Polymer-Bioaugmented PsJN Coated DAP on Wheat Growth and Yield Contributing Parameters under Field Conditions

Application of polymer-bioaugmented PsJN coated DAP improved the plant height, chlorophyll contents, grains and straw yield, total P uptake, P recovery, and agronomic efficiency (Table 3). Polymer-bioaugmented PsJN coated DAP treatment at 100% the recommended rate increased plant height of wheat (34%) compared to uncoated DAP. While, coated DAP with polymer-bioaugmented PsJN at the recommended rate resulted in a 26% increase in chlorophyll contents over uncoated DAP. Treatment with the recommended rate of polymer-bioaugmented PsJN coated DAP increased grains yield up to 23% compared to uncoated DAP. Likewise, polymer-bioaugmented PsJN coated DAP improved the straw yield compared to alone polymer coated and uncoated DAP treated treatment. PsJN bioaugmented in polymer

coated DAP at the recommended rate increased straw yield i.e., 15% and 7% compared to uncoated and alone polymer coated DAP, respectively. Polymer-bioaugmented PsJN coated DAP treatments also significantly enhanced the total P uptake by wheat crop. The 100% polymer-bioaugmented coated DAP at recommended gave 48% upsurge in total P uptake compared to commercial uncoated DAP. Phosphorus use efficiency in terms of recovery and agronomic efficiency was also improved with the application of polymer-bioaugmented coated DAP as compared to uncoated DAP. Polymer-bioaugmented PsJN coated DAP at 50% recommended rate applied plants had higher maximum P recovery and agronomic efficiency i.e., 110% and 92%, respectively, as compared to the uncoated DAP.

Table 3. Effect of polymer-bioaugmented PsJN coated DAP on yield contributing attributes of wheat in field experiment.

Treatments	Plant Height (cm)	Chlorophyll Contents (SPAD)	Grains Yield	Straw Yield	Total P † Uptake	P Recovery Efficiency	P Agronomic Efficiency
			(kg ha^{-1})				(kg grains kg^{-1} DAP)
T_1 (control)	65 f	32 f	2150 f	3333 f	9 f	_____	_____
T_2	77 d	43 de	4395 d	6400 d	27 de	20 e	25 e
T_3	83 c	48 c	4885 bc	6850 bc	33 bc	26.6 d	31 d
T_4	103 a	54 a	5400 a	7344 a	40 a	34 c	36 c
T_5	92 b	51 b	4900 b	6900 b	34 b	37 b	41 b
T_6	75 de	44 d	4300 de	6200 e	28 d	42 a	48 a
HSD	6.04	2.34	103.01	59.13	1.78	2.22	3.66

Note: Values sharing the same letter(s) did not differ significantly at 5% level of significance. T_1—control; T_2—uncoated DAP; T_3—alone polymer coated; T_4—polymer-bioaugmented PsJN coated DAP (100% of recommended rate); T_5—polymer-bioaugmented PsJN coated DAP (75% of recommended rate); T_6—polymer-bioaugmented PsJN coated DAP (50% of recommended rate), † Above ground plant biomass was used to calculate total P uptake, $n = 3$.

4. Discussion

Fertilizer diammonium phosphate (DAP) is the most important and expensive input in agricultural production [7]. It will be a great challenge to increase crop productivity in order to fulfill the food demand over the next 50 years in the presence of increasing population, decreasing arable land, rapid global climate change, increasing water scarcity, and rising price of agriculture inputs. The role of fertilizers in this scenario has gained increasing importance to get high agricultural production per unit area from the same soils, with declining soil fertility status. To meet the expected food demand without substantial increase in food price, an increase of 70–100% in production compared to present productivity is required. Furthermore, high cereal production is only possible through high inputs of fertilizers. Currently 40–60% cereal production depends upon fertilizers and by 2050, almost 110% grain production will have to depend on fertilizers [22]. Therefore, optimum utilization of chemical fertilizers in intensive cropping systems is necessary for adequate nutrient supply and optimum crop yield. Hence, it is essential to improve the applied fertilization use efficiency to achieve optimum crop yield. Over the three last decades, agronomy and genetically efficient variety has played an important role in increasing the PUE and crop productivity. Recently, new generation technologies like polymer coated P fertilizer [5] and P solubilizing bacteria and fungi are used [6], and inconsistent results were obtained due to high production cost, unsuitable microbial carrier, and poor survival at target site.

One of the essential criteria to determine the suitability of the material to be used as a carrier for inoculant is the assessment of the survival of the organism in carrier over time. Bacterial growth in the inoculant preparation is beneficial, but the viability of the population over a long time is the most important factor. Our laboratory studies results showed that among different concentrations (0.50%, 1%, and 1.5%) of polymer prototype solution, 1.5% solution sustained microbial viability up to $82 \pm 2.51 \times 10^6$ CFU^{-mL} at 30 days (Figure 1A) which was further enhanced by the addition of carbon source ($199 \pm 1.87 \times 10^{10}$ CFU^{-mL}) in selected polymer matrix {(alginate 1.5% + (1% G + 1% Gly) + PsJN)} as compared to sole addition of glucose and glycerol (Figure 1B). Microbial viability on DAP surface was also conferred when stored at different temperatures. Maximum microbe survival rate on DAP surface was estimated in sample stored at 10 °C temperature i.e., $55 \pm 2.59 \times 10^7$ CFU^{-g} fertilizers.

However, microbial viability has improved by immobilizing into suitable polymeric carriers (natural and synthetic) for prolonged time due to large surface area and its microporous structure as well as offers the possibility to form prototype or encapsulation of inoculants. Advantages related to the immobilization of microbes in polymeric matrix are conferred such as protection, slow release, prolonged storage, and easy transportation [15]. Further, addition of carbon source in polymeric formulation like disaccharides, sucrose, and trehalose as well as skimmed milk showed highly protective for microbe viability due to modification of carrier linkages for better environment, easy source of nutrition and protection under dry conditions as well as act as osmoprotectants [15,23,24].

Controlled release fertilizers are now considered an essential part of agricultural settings due to their cost effectiveness, higher efficacy, superior rate of nutrient release that matches with the crop requirement, and greater environmental friendliness [3,25]. However, this controlled release from coated fertilizers is further affected by coating material and environmental conditions [26,27]. The controlled-release P concept was also tested in the present study by comparing P release pattern in soils fertilized with commercial (uncoated), simple controlled release (alone polymer coated DAP), and polymer-bioaugmented PsJN coated on P fertilizer. Results revealed that Olsen P was decreased in soil of commercial fertilizer after 15 days but coating with and without bioaugmentation increased Olsen P up to 60 days due to controlled release pattern (Figure 2). Coating of polymer layer on DAP indicated a potential for improvement of fertilizer P efficiency and delivered P in a controlled release manner through the diffusion process from the polymer layer that allowed the plants to use it more effectively than the uncoated P fertilizer for a longer period of time [2,3,5]. The foregoing results indicated controlled release of bacterial cells from polymer-bioaugmented PsJN coated DAP in soil (Figure 1D). Therefore, the time-course profile for cell release showed that at 15- and 60-day incubation linear increase in viable cell number, the final cell concentration in the soil was up to $77 \pm 3.32 \times 10^6$ CFU^{-g} soil. After application, soil polymeric structure becomes weak or breakable by attack of indigenous soil microbes and enzymes produced by entrapped microbes which cause gradual discharge from the polymeric carrier [14]. These microbes not only improved the crop yield by different mechanisms but also solubilized the fixed P by producing various organic acids and other activities [6,11]. It is well reported that microbial inoculation results in enhanced crop growth and adaptation to local conditions especially under diverse agricultural settings [28].

The enhancement of growth, yield, and nutrients concentration in wheat crop was due to dual benefit of coated DAP [29] and successful delivery of microbes at the target site which improved growth and yield contributing parameters of wheat crop via growth promotion mechanisms including hormone production, nutrient solubilization, rhizosphere modification evoked various physiological processes to help the plants to sustain growth [6,11]. These findings are correlated with those previously reported [30,31].

The control release mechanism of nutrient and microbes improved the nutrients availability by the mechanism of consistent/slow release of microbes and fertilizer and solubilization of fixed P. This availability improved the plant nutrient absorption and nutrient use efficiency as result increased the growth and yield [3,5,15,16]. These findings are further substantiated with Fageria et al. [32] where they reported 25% increase in NUE due to polymer coated urea application. The enhancement of P use efficiency might be related to lower fixation of P on soil components that would otherwise increase using conventional fertilizers [33]. Moreover, recently, Imran et al. [34] reported increased PUE and growth and yield of wheat under the application of glycerin and polymer coated phosphatic fertilizer in calcareous soil conditions.

In present study, another potential mode of action of high nutrients availability is the presence of ammonia in the DAP fertilizer which upon oxidation caused acidification of rhizosphere with importance for Ca-P solubilization but also for mobilization of micronutrients such as Fe, Zn, Mn, and Cu in high pH soils. These effects may be even amplified due to the localized application and stabilization of ammonium in encapsulated granules, which has been shown to mediate P solubilization and local root growth proliferation even at soil pH levels around 8 [35]. Moreover, more recent studies demonstrated that these are conditions promoting the root colonization by various plant growth-promoting microorganisms [36–38].

Additionally, various studies demonstrated ammonium-induced stimulation of auxin production by various PGPR strains [39–41] finally leading to improved root growth (Table 1). Consequently, a larger root system with ammonium-induced rhizosphere acidification promoted P and micronutrient mobilization but also nutrient acquisition in general [39]. Table 2 supports this scenario showing that the polymer-bioaugmented DAP treatment increased not only the plant P status but also the N and K concentrations, as compared with the non-inoculated variants.

It can be concluded that polymer proved a good carrier for microbes and polymer entrapped microbes coated DAP fertilizer application increased available P by consistent release and reduction of fixation and solubilizing the fixed P according to plant requirement and growth stages, which finally results in increased growth and yield of wheat. Figure 4 shows that the relationship between wheat growth stages and maximum P requirement also clearly explained the potential of different coated DAP fertilizer to provide P according to plant requirement. Phosphorus requirement of wheat over the growing season is best explained by sigmoid curve. At tillering (25 days after germination), wheat plants only uptake total P up to 35% but rapid P uptake occurs during stem elongation and heading where its uptake reaches 80–100% complete. Uncoated DAP fertilizer released maximum P in soil during 15 days of incubation after that decline start which is likely to fix in soil under field conditions under a high concentration of P reacting cations. By using the commercial uncoated DAP fertilizer, there is no adequate amount of P available to wheat at crucial stages. Furthermore, the experiment was conducted under pot and field conditions to check the efficacy of coated and uncoated DAP fertilizer on growth, yield, and P use efficiency of wheat. Significantly increased wheat growth, yield, and PUE over commercial uncoated DAP was found, as consistent release of P from coated fertilizer made P available for uptake. Moreover, reduced rates of coated fertilizer at 50% of the recommended rate gave better results as compared to the recommended rate of uncoated DAP. Overall, results of this research work suggest the use of coated DAP fertilizer (efficient fertilizer) at the recommended rate as well as reduced rates at 75% and 50% of the recommended rate over uncoated DAP fertilizer without compromising grain yield of wheat.

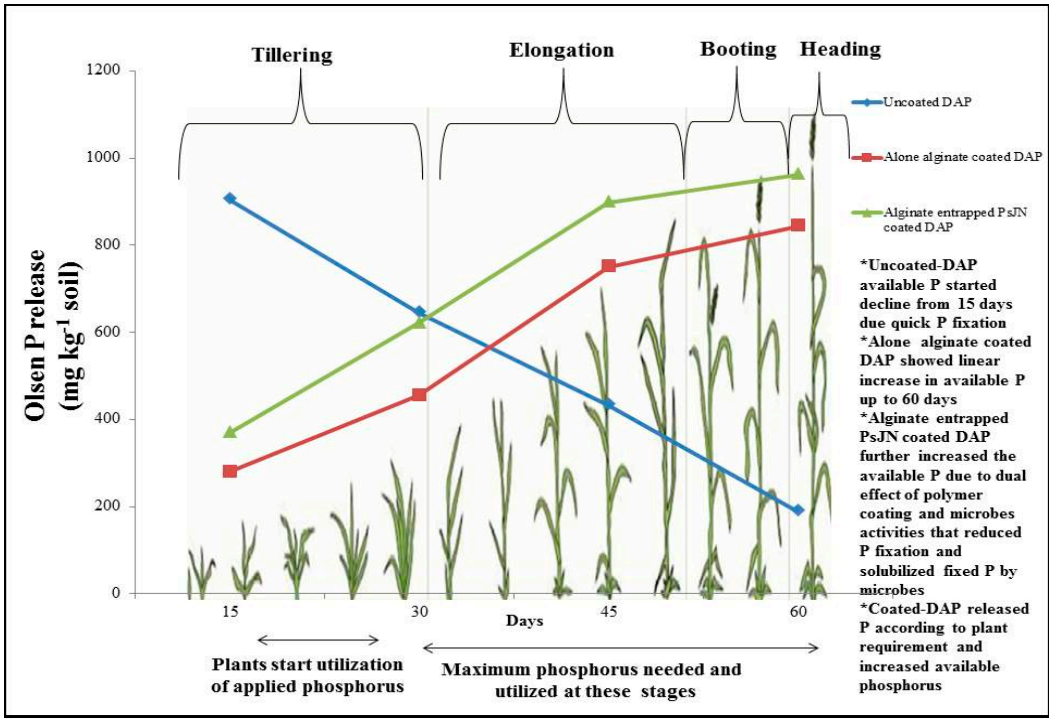


Figure 4. The phosphorus release in soil from alginate coated and uncoated DAP fertilizer over different time intervals and growth stages of wheat.

5. Conclusions

The viability of PsJN increased as a function of protective property of polymeric formulation, declined with reducing polymer (alginate) concentration, cell survival rate further increased by addition of carbon source in polymer matrix and showed no/little effect of storage temperature on cell viability on polymer-bioaugmented PsJN coated DAP surface. Controlled release of P and microbes from coated DAP reduced P fixation and enhanced the available P for plant uptake. Based on the findings of the present study it can be concluded that alginate bioaugmented DAP approach could be very effective in enhancing growth, yield, and phosphorus use efficiency of wheat. Alginate bioaugmented DAP even at a 75% rate had more yield under field condition compared to uncoated DAP at 100% rate. Thus, alginate bioaugmented DAP could be an innovative strategy for improving growth, yield, and nutrient use efficiency of wheat. However, as a perspective, there is a need to assess the potential of alginate bioaugmented DAP under different agro-ecological zones in the future.

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